New Neurotransmitter Synthesis Mimicking Serotonin

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

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To whome it may concern

This is to certify that Miss. Sonia Sinha, pursuing a M.Sc. in Biotechnology, Sister Nivedita University, Kolkata, has successfully completed her internship under our guidance from January, 2025 to May, 2025 at Institute of Health Sciences, Presidency University, Kolkata, on ''New neurotransmitters synthesis mimicking serotonin''.

Sonia is very sincere, punctual, disciplined and obedient. We have always noticed a great deal of determination in Sonia and she is an enthusiastic student who always strives for new knowledge. We wish her every success in life.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "NEW NEUROTRANSMITTER SYNTHESIS MIMICKING SEROTONIN" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from 12th January 2025 to 12th May 2025 under the supervision of Dr. Arpita Konar and Dr. Piyali Sarkar, Department of Institute of Health Science, Presidency University. I acknowledge that I have read and understood the UGC regulations, 2018 (*Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions*). I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software, " Small SEO Tool". At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.

Sonia Sinha
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TO WHOM IT MAY CONCERN

This is to certify that the project report titled "NEW NEUROTRANSMITTER SYNTHESIS MIMICKING SEROTONIN", submitted by Ms Sonia Sinha (Registration No. 230020160833 Enrolment No. 2322210002016) to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfillment of the requirements for the Master of Science degree.

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I am especially grateful to my supervisor, Dr. Arpita Konar. Her guidance, expertise, and unwavering support have been crucial in shaping the direction of my research. The insightful feedback she provided and her commitment to academic excellence have inspired me to expand the boundaries of my understanding.

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ABBREVIATIONS

ABBREVIATIONS	MEANING
NMR	Nuclear Magnetic Resonance
CNS	Central Nervous System
DMEM	Dulbecco's Modified Eagle Medium
GABA	Gamma Aminobutyric Acid
RBF	Round-bottom flask
TLC	Thin Layer Chromatography
DMSO	Dimethyl Sulfoxide
PBS	Phosphate Buffered Saline
EDTA	Ethylenediamine tetraacetic acid
FBS	Fetal Bovine Serum
RNA	Ribonucleic Acid

ABSTRACT

Neurotransmitters are essential chemical messengers within the body's nervous system, responsible for facilitating communication between neurons and their target cells. These biomolecules play a critical role in regulating a wide array of physiological processes, including the heartbeat, digestion, mood regulation, and muscle movement. Given their importance in maintaining homeostasis and enabling brain function, neurotransmitters are a focal point in both neuroscience and pharmacological research. The increasing global emphasis on sustainability and environmental responsibility has prompted researchers to explore green chemistry approaches for synthesizing biologically significant molecules, including neurotransmitters. This dissertation investigates the green synthesis of neurotransmitters and their functional analogs, emphasizing the use of environmentally benign reaction conditions to minimize ecological harm and the generation of toxic byproducts. By leveraging innovative techniques, natural reducing agents, and mild reaction parameters, the study aims to develop more sustainable pathways for neurotransmitter production. Comprehensive characterization of the synthesized compounds was performed using Nuclear Magnetic Resonance (NMR) spectroscopy to confirm their structural integrity and ensure their biological relevance. Once characterized, these compounds are subjected to cell-based assays, such as the MTT assay, to evaluate their bioactivity and potential therapeutic effects. The research also explores the broader applications of green-synthesized neurotransmitters in neuropharmacology and biosensing, highlighting their enhanced compatibility with biological systems and improved therapeutic efficacy compared to traditionally synthesized counterparts. Ultimately, this work demonstrates that green synthesis not only offers a viable alternative to conventional production methods but also fosters more sustainable advancements in neurochemistry and biomedical research. By reducing environmental impact and improving the safety profile of neurotransmitter synthesis, green chemistry is paving the way for innovative solutions in neuroscience and drug development.

Keywords: Neurotransmitters; Serotonin; Green Synthesis; Blood Brain Barrier; Eco friendly; Pharmacophore.

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INTRODUCTION

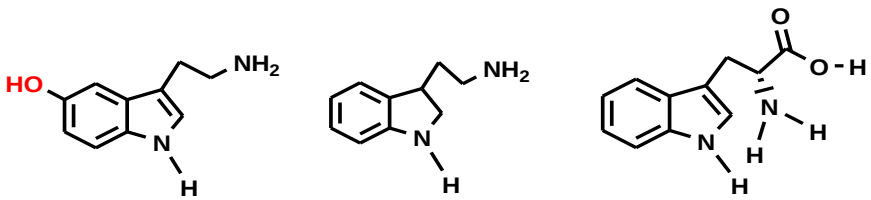
Neurotransmitters are chemical substances that play an important role in transmitting signals across synapses, enabling communication between neurons and their target tissue sand that are synthesized in neurons as a result for some of its precursor substances to go through an enzymatic process [2]. Depending on the type of neurotransmitter, either the cell body or the presynaptic terminal may act as the production [1][3]. An example is the conversion of the amino acid tyrosine to dopamine, which involves enzymatic processes by tyrosine hydroxylase and aromatic L-amino acid decarboxylase, resulting in excitation, inhibition, or functional modification [3]. The Central Nervous System (CNS) utilize neurotransmitters to regulate various sensory, motor, and integrative neuronal messages, thereby affecting various bodily functions such as emotions, thoughts, memories, movements, and sleep patterns [2] Until now, scientists have identified over 100 neurotransmitters, with some of the most significant ones being acetylcholine, norepinephrine, dopamine, gamma-aminobutyric acid (GABA), glutamate, serotonin, and histamine [2][3]. Based on the functional effect, these chemical messengers can be broadly classified Some major types are: excitatory, inhibitory, and modulatory neurotransmitters. For example, excitatory neurotransmitters like glutamate stimulate the transmission of signals, while inhibitory neurotransmitters like GABA and glycine suppress signal transmission and lastly, Modulatory neurotransmitters, such as dopamine and serotonin, that influence the actions of other neurotransmitters and are essential for managing a number of physiological processes. Dysregulation of neurotransmitter activity has been implicated in various neurological and psychiatric disorders, like Alzheimer's disease, Parkinson's disease, depression, anxiety disorders, and schizophrenia. One such important neurotransmitters is Serotonin, which found in the brainstem and gastrointestinal tract [3]. Serotonin has various complicated role in mood regulation, sleep-wake cycles, appetite, memory, temperature regulation, pain perception, and emotional response. Serotonin is an inhibitory neurotransmitter and tends to cause calmness by the reduction of neuronal excitability. But there are some problems associated with serotonin, such as Serotonin which is large, polar molecule which have molecular weight approximately 176 Daltons, and contains multiple hydroxyl(-OH) groups, which contribute to its polarity, on the other side Blood Brain Barrier is designed to allow only small, non-polar or lipophilic molecules to pass through easily, hence serotonin cannot diffuse across the lipid bilayer of endothelial cells that form the Blood Brain Barrier [4][5]. Along with other limitation, including lack of selectivity, poor bioavailability, and various side effect. This research aimed to address these challenges by developing a Pharmacophore that could more effectively mimic serotonin's effects. The primary compounds utilized in this reaction was isatin and amines as starting materials.

AIMS AND OBJECTIVES

I. To develop Environmentally friendly, safe and effective therapeutic agents that can enhance neurotransmitter function or mimic their action in the treatment of neurodegenerative diseases,
   - one aspect of my research is to synthesis by using green chemistry approach, emphasizing environmental sustainability
   - This method helps in minimizing chemical waste and need for extensive purification steps.
   - During the reaction copper is used as a catalyst, which was recovered and reused, promoting resource conservation and sustainable practice.

II. To Design and synthesize a Pharmacophore that can effectively cross blood brain barrier.

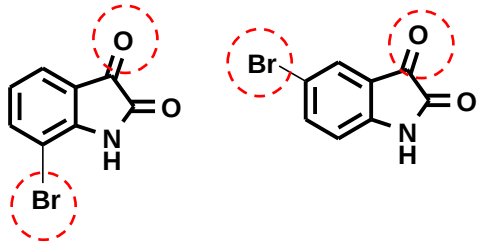
DESIGNING ASPECT



Serotonin Tryptamine D-Trytophan

Figure 1: Structures of Serotonin, Tryptamine and D-Tryptophan

Serotonin's structure includes an indole ring system, to this indole core a hydroxyl group (-OH) is attached at carbon in the 5-position [6]. In contrast, tryptophan, an essential amino acid in the body, that serves as a precursor to serotonin, and Tryptamine is an aminoalkylindole consisting of indole having a 2-aminoethyl group at the 3-position, can easily cross the Blood Brain Barrier. Therefore, comparing the structure of serotonin with tryptophan and tryptamine compounds gives a hint that the presence of the hydroxyl group may hinder its ability to cross the Blood Brain Barrier [7].



7-Bromo Isatin 5-Bromo Isatin

Figure 2: Actives Sites of Bromo Substituted Isatin Moiety

For this thesis, we have chosen Two Bromo-Substituted Isatin Moiety (7-bromo Isatin and 5-Bromo Isatin) which have an indole ring (similar structure like serotonin) and two active centers for amine incorporation, one is Bromide center and another one is Ketone center which are shown in Figure 2. And amines that will react respectively are N, N-Dimethylaniline and 4-picolylamine. These selected amines are expected to form a stable product, that further modifies the original indole structure upon reaction with the Isatin Moieties thus enhancing the potential to cross the Blood Brain Barrier.

MATERIALS AND METHOD

I. COMPOUND A:

- **CHEMICALS AND REAGENTS**: 7-Bromoisatinn (7-bromo-1H-indole-2,3-dione); N, N-Dimethylaniline; Copper Nanoparticles; Distilled Water; Silica Gel; Hexane; Ethyl Acetate; Methanol; Chloroform.
- **EQUIPMENT AND APPARATUS**: Round Bottom Flask; Magnetic Beads; Spatula; Condenser; Magnetic Stirrer (or Magnetic Rotary Stirrer); Voltage Regulator; Tullu Pump; Silicone Oil Bath; Rotary Evaporator; Chromatography Columns; Funnels; Conical.

- **REACTION:**

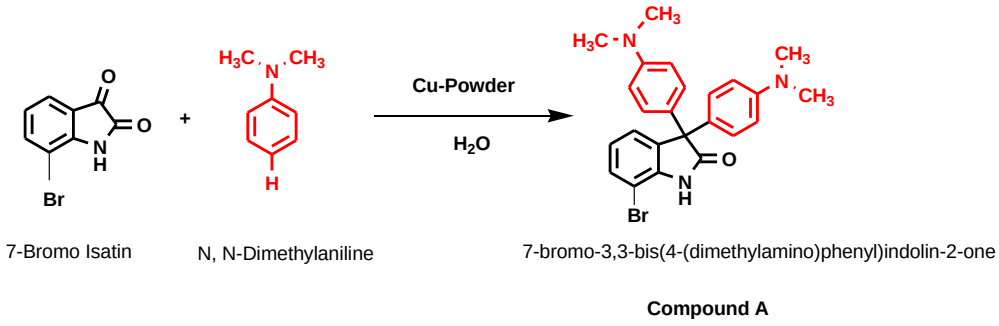


Figure 3: Reaction of 7-Bromo Isatin and N, N-Dimethylaniline

- **PROCEDURE:**

Weighing Chemicals: Using weighing machine 1.5 mmol of 7-Bromoisatin which weigh about 339 mg is taken and using pipette we took 3 mmol of N, N-Dimethylaniline which measured about 375 µl.

Reaction Setup: Weighed chemicals which are carefully measured out are then transferred into a round-bottom flask, along with the catalyst, copper powder and Distilled water. Following

the preparation of the mixture, the round-bottom flask containing the chemicals, copper nanoparticles, and distilled water is set up for heating under reflux conditions which involves connecting the round-bottom flask to a condenser. .

The round-bottom flask, which is connected to the condenser, is then placed in a silicon oil bath which is kept above Magnetic Stirrer Plate which provides continuous mixing and the oil bath is connected to a temperature regulator, which is used to maintain the temperature of the oil bath at a specific, constant value. In this procedure, the temperature is regulated around 120°C. The reaction is continued 48 hours.

Crude Preparation (Workup): After 48 hours when the reaction is completed, the remaining water in the RBF is completely evaporated using rotary evaporator. After drying, we perform an extraction to separate the product from solid copper nanoparticles. For separation, we pour a polar solvent such as ethyl acetate, methanol, or chloroform. After adding the solvent, we keep the RBF still in the holder and then pour off the upper layer, separating it from the lower sediments of copper nanoparticles, into an Evaporating dish. We repeat this process 3 to 4 times and later add silica gel to create a slurry and leave it until it gets dry and form powdery.

Column Chromatography Setup and Purification: Once the column is cleaned, we secure it vertically using a ring stand. Next, to pack the stationary phase, we suspend silica gel in hexane and pour the slurry into the column until we reach desired hight. Once it reached, we load our sample onto the top of the packed column. Once the column is ready, open the pinch clamp (or stopcock) to allow the mobile phase to flow through it slowly, maintaining constant wetness in the column. The eluates are collected in conical flasks, which are then poured into a round-bottom flask. The collected eluate is then evaporated using a rotary evaporator, leaving the solid or liquid product behind. After repeating the elute collection process at a particular solvent percentage for some time, the solvent percentage is gradually increased and it's continued until we start getting solid or liquid product.

Monitoring Progress of the Reaction: To monitor the progress of the reaction, the product is mixed with a solvent such as ethyl acetate or methanol. A small capillary is then used to place a small dot on a TLC plate, which is subsequently kept in a mobile phase jar. Finally, the plate is examined under UV light to determine whether the desired compound has been separated.

Sample collection: The solid or liquid product that we obtained is then collected in culture tubes and kept for several days in a dark place to allow for crystal formation. A few crystals are then collected in a tube for further study of the product's structure. The remaining crystals are weighed and dissolved in Dimethyl Sulfoxide (DMSO) for cell testing.

CELL TESTING OF COMPOUND A

- **CELL LINE DESCRIPTION:** SH-SY5Y cells, derived from are the neuroblastoma cell line which were subcloned from the parental SK-N-SH cell line, which originated from a bone marrow biopsy of a four-year-old female [8]. These cells display a neuroblast-like

morphology, growing as both adherent and floating clusters, and can be differentiated into neuron-like cells using compounds such as retinoic acid and neurotrophins [9][10]). SH-SY5Y cells are used worldwide as an in-vitro model for neuronal function and differentiation, they can be characterized by their ability to proliferate in culture, express immature neuronal markers in the undifferentiated state, and upon differentiation, exhibit mature neuronal markers and cease proliferation [10][11]. Their sensitivity to culture conditions and slow proliferation rate requires careful handling, but their biochemical similarity to human dopaminergic neurons and versatility in experimental neuroscience make them a key tool for research [10].

o METHODOLOGY

Thawing: SH-SY5Y cells were retrieved from cryopreservation, for which the frozen vial was quickly removed from liquid nitrogen and placed immediately in a 37°C water bath. Gentle agitation was applied to ensure rapid and even thawing, taking care to remove the vial as soon as the contents were completely liquid to minimize exposure to potentially cytotoxic DMSO. Then the thawed cells were transferred into a sterile conical tube containing growth medium. After 24 hours, the medium was replaced to remove non-viable cells and any remaining DMSO, ensuring optimal cell health and attachment.

Splitting of SH-SY5Y Cells: After 2 to 3 days the old growth medium was aspirated, and the cell monolayer was gently rinsed with 1X PBS to remove residual serum and cell debris. After aspirating the PBS, 500ul-1ml 0.25% trypsin-EDTA solution was added to the flask. The flask was incubated at 37°C for 2–3 minutes, with periodic gentle tilting to aid cell detachment. The cell suspension was triturated gently with a pipette to ensure a single-cell suspension and then centrifuged it at 1000g for 5 minutes. The supernatant was removed, and the cell pellet was resuspended in 1ml DMEM with 10%FBS supplement. 10ul of the solution and 50ul of trypan blue was added to make 6X dilution. 10ul of the solution was taken Then the cells counted using a haemocytometer.

Freezing of SH-SY5Y Cells: Cells when reached to 85-95% confluency, were aliquoted into cryovials at a density of 2–6 million cells per vial. The cryovials were pre-cooled at 4°C for 10 minutes, then placed at –20°C for an additional 10 minutes to allow gradual temperature reduction. The vials were then transferred to a –80°C freezer for 24 hours before being moved to liquid nitrogen for long-term storage.

Cell Seeding and Treatment: In 96-well plate, they were seeded around 10,000 to 20,000 cells per well. Once the cells reached a desired confluency of around 50-60%, Cells in 6-well plates were divided into 4 groups:

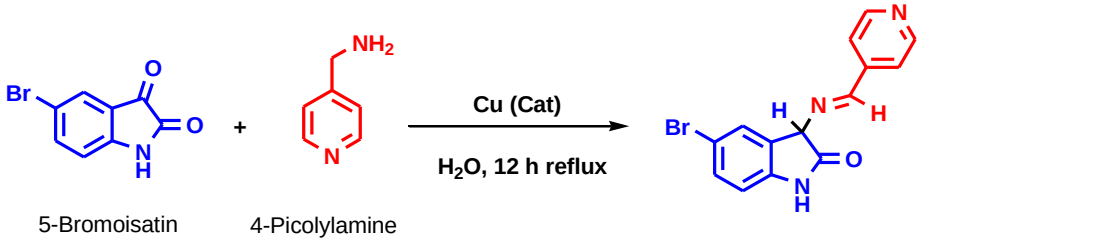
- Naive control [only media change without FBS],
- Vehicle Control [0.1% DMSO in the DMEM without FBS],
- 1 µM of Compound A [DMEM without FBS], and
- 10 µM of Compound A [DMEM without FBS]. (The use of serum-free media (DMEM without FBS)

After 48 hours of the treatment, 10 µl of MTT solution (5 mg/ml) was added to each well (final conc. 0.45 mg/ml). The solution was then incubated for 1-4 hrs at 37° C. 100 µl DMSO was added to each well for dissolving the formazan crystals. The solution was mixed properly to ensure the complete solubilization. Absorbance was measured at 570 nm.

II. COMPOUND B:

- **CHEMICALS AND REAGENTS:** 5-Bromoisatin; 4-Picolylamine; Copper Nanoparticles; Distilled Water; Silica Gel; Hexane; Ethyl Acetate; Methanol; Chloroform.
- **EQUIPMENT AND APPARATUS:** Round Bottom Flask; Magnetic Beads; Spatula; Condenser; Magnetic Stirrer (or Magnetic Rotary Stirrer); Voltage Regulator; Tullu Pump; Silicone Oil Bath; Rotary Evaporator; Chromatography Columns; Funnels; Conical.

- **REACTION:**



5-Bromoisatin 4-Picolylamine

(*E*)-5-bromo-3-((pyridin-4-ylmethylene)amino)indolin-2-one

Compound B

Figure 4: Reaction of 5-BromoIsatin and 4-Picolylamine

- **PROCEDURE:**

Weighing Chemicals: 1.5 mmol of 5-Bromoisatin is weighed about 329 mg is taken and using pipette we took 3 mmol of 4-Picolylamine which measured about 375 µl.

Reaction Setup: Weighed chemicals which are carefully measured out are then transferred into a round-bottom flask, along with the catalyst, copper powder and Distilled water. Following the preparation of the mixture, the round-bottom flask containing the chemicals, copper nanoparticles, and distilled water is set up for heating under reflux conditions which involves connecting the round-bottom flask to a condenser. .

The round-bottom flask, which is connected to the condenser, is then placed in a silicon oil bath which is kept above Magnetic Stirrer Plate which provides continuous mixing and the oil bath is connected to a temperature regulator, which is used to maintain the temperature of the oil bath at a specific, constant value. In this procedure, the temperature is regulated around 120°C. The reaction is continued 12 hours.

Crude Preparation (Workup): 12 hours after the reaction is over the remaining water in the RBF is completely evaporated using rotary evaporator**.** After drying, we perform an extraction to separate the product from solid copper nanoparticles. For separation, we pour a polar solvent such as ethyl acetate, methanol, or chloroform. After adding the solvent, we keep the RBF still in the holder and then pour off the upper layer, separating it from the lower sediments of copper nanoparticles, into an Evaporating dish. We repeat this process 3 to 4 times and later add silica gel to create a slurry and leave it until it gets dry and form powdery.

Column Chromatography Setup and Purification: Once the column is cleaned, we secure it vertically using a ring stand. Next, to pack the stationary phase, we suspend silica gel in hexane and pour the slurry into the column until we reach desired hight. Once it reached, we load our sample onto the top of the packed column. Once the column is ready, open the pinch clamp (or stopcock) to allow the mobile phase to flow through it slowly, maintaining constant wetness in the column. The eluates are collected in conical flasks, which are then poured into a round-bottom flask. The collected eluate is then evaporated using a rotary evaporator, leaving the solid or liquid product behind. After repeating the elute collection process at a particular solvent percentage for some time, the solvent percentage is gradually increased and it's continued until we start getting solid or liquid product.

Monitoring Progress of the Reaction: To monitor the progress of the reaction, the product is mixed with a solvent such as ethyl acetate or methanol. A small capillary is then used to place a small dot on a TLC plate, which is subsequently kept in a mobile phase jar. Finally, the plate is examined under UV light to determine whether the desired compound has been separated.

Sample collection: The solid or liquid product that we obtained collected for a Nuclear Magnetic Resonance or NMR tube for further study of the product's structure.

RESULTS AND DISCUSSIONS

I. COMPOUND A:

- **Characterization of compound [A] using ^{1}H-NMR (600MHz):**

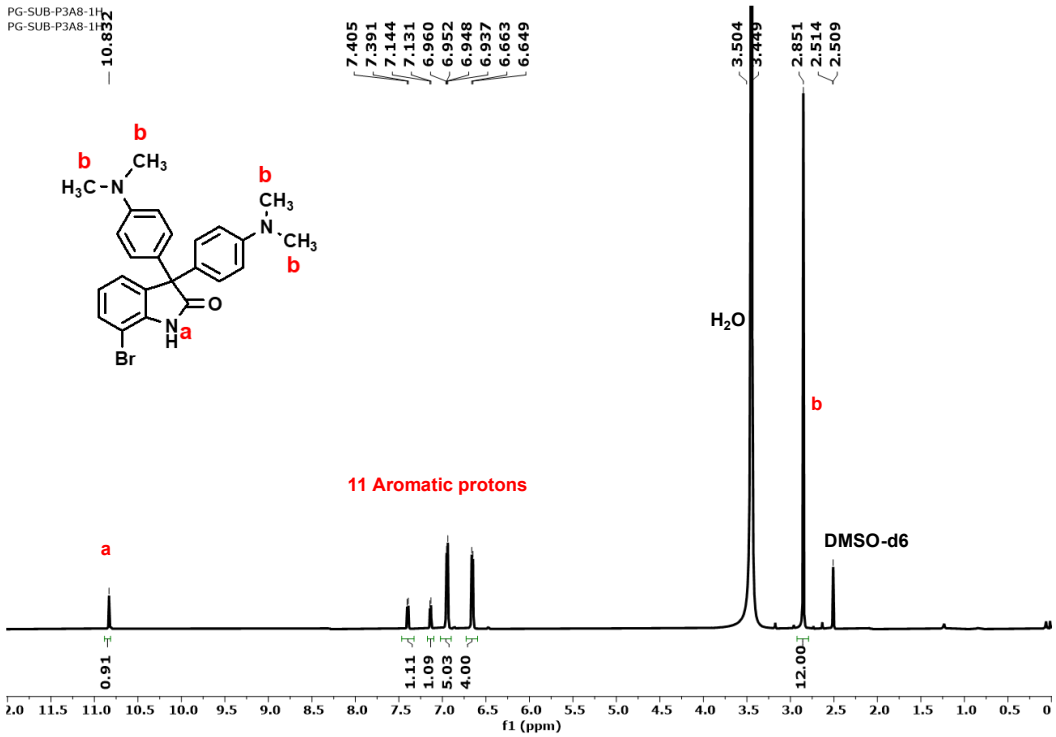


Figure 5: ^{1}H NMR spectrum of Compound A

The ^{1}H NMR spectrum provides information:

- **Aromatic Region**: A complex series of multiplets is observed between approximately δ 6.6 ppm and 7.5 ppm. his region is characteristic of aromatic protons. The molecule has an indole ring (containing 4 aromatic protons), a brominated benzene ring (containing 3 aromatic protons), and two N-methylated benzene rings (each containing 2 aromatic protons due to symmetry).
- **Peak "a":** A signal is observed around δ 10.80 ppm, integrating approximately 1H. This chemical shift is characteristic of an amide proton (N-H).
- **Peak "b":** A sharp singlet is observed at approximately δ 3.49 ppm, with a large integration of 12.00 H. This chemical shift represents the presence of aliphatic protons.

- **Characterization of compound [A] using 13C-NMR (150MHz):**

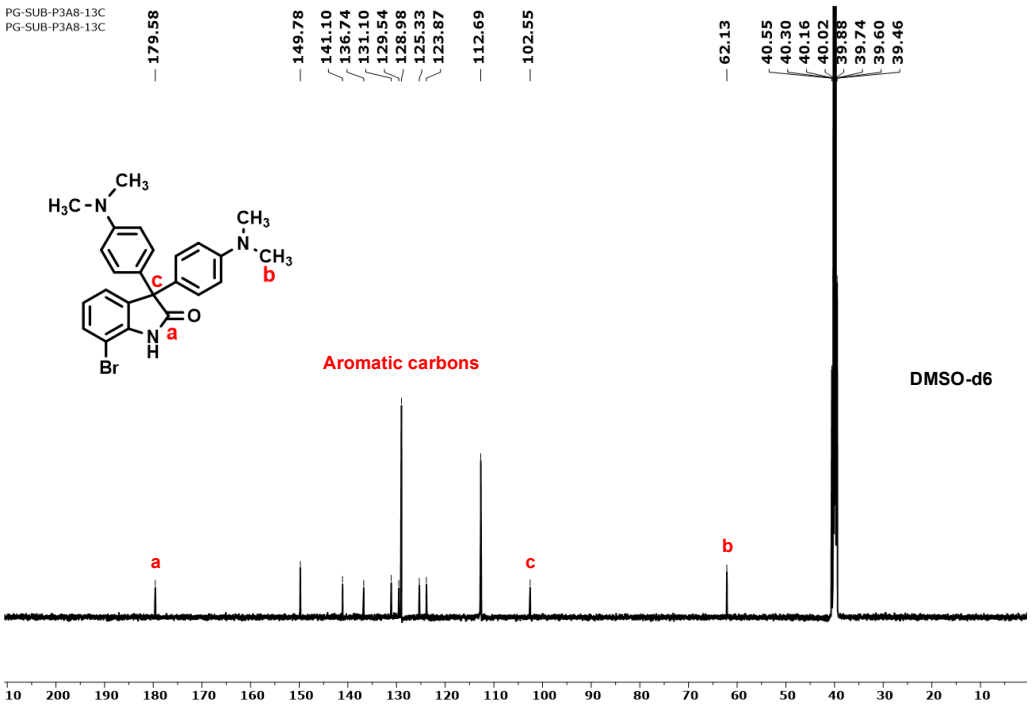


Figure 6: ^{13}C NMR spectrum of Compound A

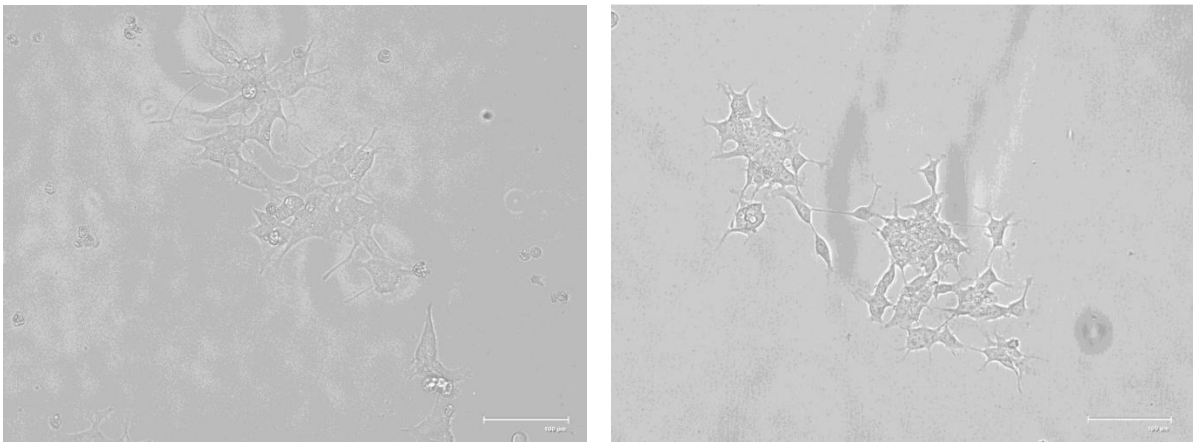
The ^{13}C NMR spectrum provides information:

- **Carbonyl Carbon** (Peak "a"): A singlet at δ 179.58 ppm: This signal is significantly downfield and is characteristic of a carbonyl carbon (C=O).
- **Aromatic Carbons**: A cluster of signals appears in the region between approximately δ112.60 ppm and 149 ppm. This region is typical shows presence for aromatic carbons.
- **Peak "c"**: It represent a quaternary carbon.
- **N-CH_3 Carbons** (Peak "b"): A peak at 62.10 ppm shows a chemical shift suggests a carbon attached to a nitrogen atom in one of the N-methylated benzene rings.

- **RESULT OF CELL TESTING:**

In the Figure 7 bellow, marked (i) is the Control; (ii) is the vehicle control; and in the Figure 8 bellow, marked (iii) is with 100nM concentration of compound A; (iv) is with1µM concentration of compound A. The results from the cell testing showed a strong formation of neuronal networks and an increase in synaptic neurons across four different setups: Control, Vehicle Control, one with a 100nM concentration, and another with a 1µM concentration of compound A after 48 hours. Thus, it shows a positive effect on neural connectivity and function. Observing well-formed neuronal networks is essential for evaluating the neurodevelopmental effects of compounds in vitro. At the same time, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a well-known method for measuring cell metabolic activity and viability, confirmed that Compound A is

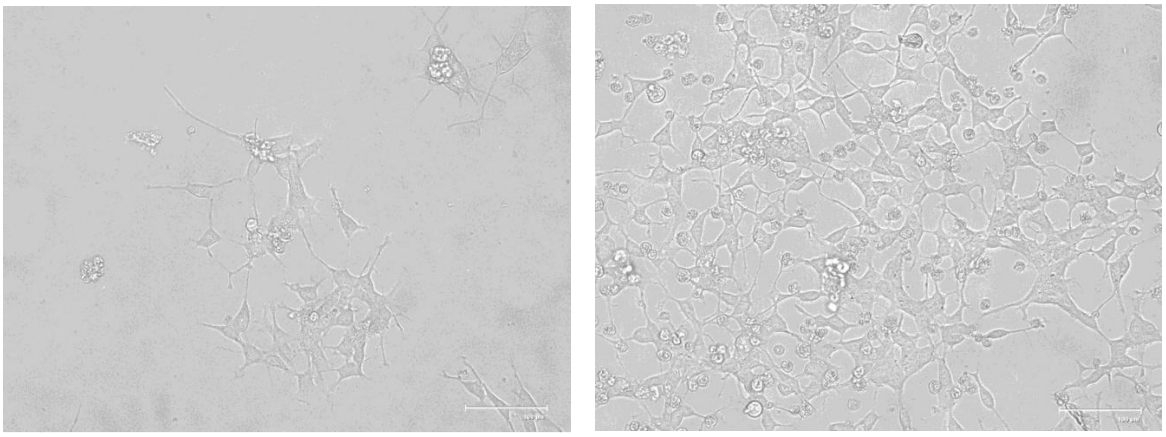
not cytotoxic. With these promising in vitro results, Compound A is considered ready for further in vivo studies. These studies will shed light on its pharmacokinetics, pharmacodynamics, and overall therapeutic potential in a living organism



(i) Control (ii) Vehicle Control

Figure 7: Microscopic Images of SH-SY5Y neuroblastoma cells after 48 hours

(i) In Control setup: Cells were subjected to media change only. After 48 hours they are still exhibiting Normal Morphology. (ii) Vehicle Control: After 48 hours the appearance of these cells seems similar to control, indicating the presence of DMSO does not have a significant effect on cell morphology



(iii) 100nM concentration (iv) 1µM Concentration

Figure 8: Microscopic Images of SH-SY5Y cells 48 hours after treatment with Compound A.

Synthesized Compound A was tested on SH-SY5Y Cells at two different concentrations of (iii) 100nM and (iv) 1µM Concentration. At both Concentrations, the cell, induced significant neuronal network formation and extensive neurite outgrowth, similar to the effects typically observed with serotonin.

II. COMPOUND B:

- **Characterization of compound [B] using ^{1}H-NMR (600MHz):**

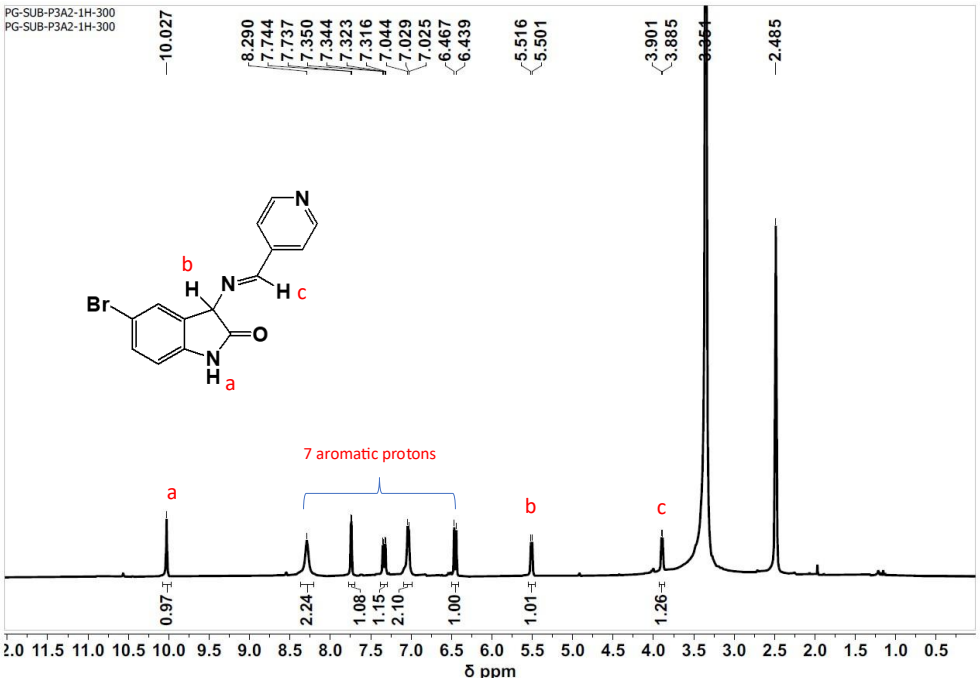


Figure 9: ^{1}H NMR spectrum of Compound B

The ^{1}H NMR spectrum provides information:

- **Aromatic Region**: A cluster of signals between δ 6.4 and 8.3 ppm. The integrated region shows around 7 aromatic protons.
  - The signals at δ 8.290, 7.744, 7.737, 7.344, 7.323, 7.316 ppm likely correspond to the protons on the pyridyl ring (the six-membered ring containing nitrogen).
  - The signals at δ 7.029, 7.025, 6.467 ppm likely correspond to the protons on the bromo-substituted indole ring system.
- **Peak "a":** A broad signal at δ 10.03 ppm. This chemical shift is characteristic of an indole N-H proton, integrating one proton, consistent with the single N-H in the indole ring
- **Peak "b":** A signal is observed at approximately δ 5.52 ppm, with an integration of 1H. This chemical shift suggests a proton attached to a carbon that is bonded to an electronegative atom, like nitrogen. This is a methine proton (C-H) on the indole ring adjacent to the nitrogen and the bromine-substituted benzene part.
- **Peak "c":** A signal is observed at approximately δ 3.90 ppm, with an integration approximately 1H. This chemical shift also suggests the presence of Ethylenic protons.

- **Characterization of compound [B] using 13C-NMR (150MHz)**

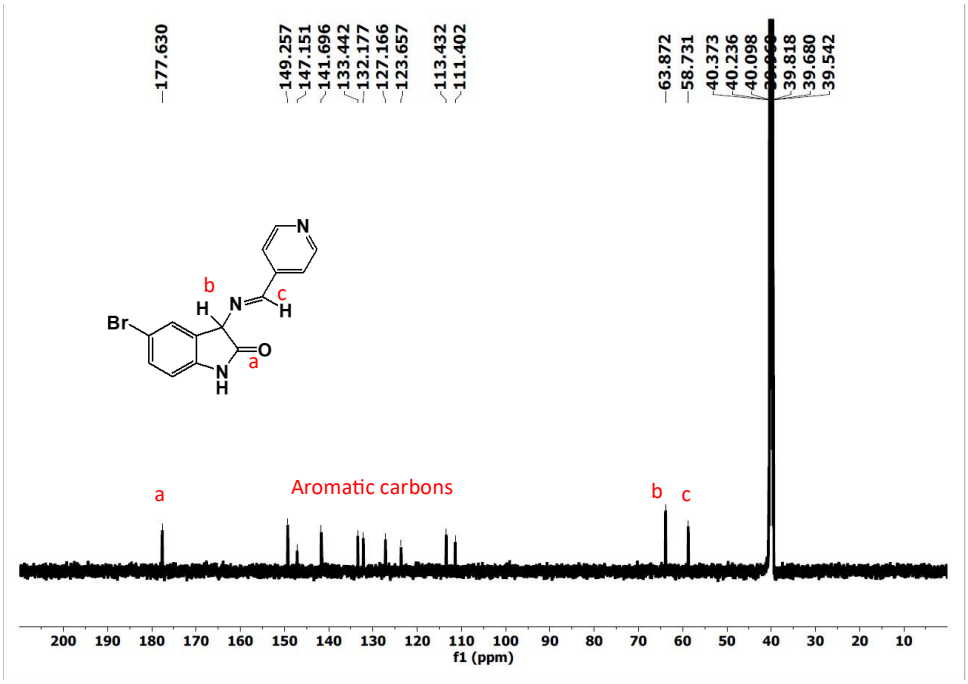


Figure 10: ^{13}C NMR spectrum of Compound B

The ^{13}C NMR spectrum provides information:

- **Carbonyl Carbon** (peak a): A prominent peak is visible at δ 177.630 ppm: This signal is significantly downfield and is characteristic of a carbonyl carbon (C=O).
- **Aromatic Carbons**: The cluster of signals between 111 ppm and 150 ppm corresponds to the carbon atoms in the two aromatic rings (the bromo-substituted indole and the pyridyl ring). Each unique carbon atom in these rings will give rise to a separate signal. The number of signals in this region (11 distinct peaks are clearly visible) is consistent with the 11 non-equivalent carbon atoms in the aromatic system.
- **Aliphatic Carbons (**peak b and peak c**)**: Two Peaks around δ 63.872 ppm and δ 58.731 ppm indicates the presence of Aliphatic carbon.

CONCLUSION:

For Compound A, the combined 1H and 13C NMR data confirm the compound a is 7-bromo-3,3-bis(4-(dimethylamino)phenyl)indolin-2-one. Further cell-testing demonstrate that Compound A promotes the formation of neuronal networks and increases synaptic neuron counts, indicating a positive effect on neural connectivity and function and the MTT assay further confirms that Compound A is not cytotoxic.

For Compound B, the combined 1H and ^{13}C NMR data confirm the compound B is (E)-5-bromo-3-((pyridin-4-ylmethylene)amino)indolin-2-one.
With this I would like to conclude that the both compounds have been thoroughly characterized at the molecular level, and Compound A has shown promising primary biological activity enhancing neuronal network formation without cytotoxicity. Therefore, these findings provide a strong base for advancing both compounds to further studies, including in vivo evaluation of their pharmacokinetics, pharmacodynamics, and therapeutic potential in neurodevelopmental and neuroprotective contexts.

REFERENCES

1. Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Neurotransmitter Synthesis.
2. Sheffler ZM, Reddy V, Pillarisetty LS. Physiology, Neurotransmitters. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539894/
3. Nagatsu T. (2000). *Rinsho shinkeigaku = Clinical neurology*, *40*(12), 1185–1188.
4. Du, W., Chen, H., Gróf, I. *et al.* Antidepressant-induced membrane trafficking regulates blood-brain barrier permeability. *Mol Psychiatry* **29**, 3590–3598 (2024). https://doi.org/10.1038/s41380-024-02626-1
5. Becker, G., Da Silva, S., Sabo, A. N., Antal, M. C., Kemmel, V., & Monassier, L. (2021). Blood-Brain Barrier Permeability: Is 5-Hydroxytryptamine Receptor Type 4 a Game Changer?. *Pharmaceutics*, *13*(11), 1856. https://doi.org/10.3390/pharmaceutics13111856
6. Barik S. (2020). The Uniqueness of Tryptophan in Biology: Properties, Metabolism, Interactions and Localization in Proteins. *International journal of molecular sciences*, *21*(22), 8776. https://doi.org/10.3390/ijms21228776
7. Höglund, E., Øverli, Ø., & Winberg, S. (2019). Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review. *Frontiers in endocrinology*, *10*, 158. https://doi.org/10.3389/fendo.2019.00158
8. Ross, R. A., Spengler, B. A., & Biedler, J. L. (1983). Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *Journal of the National Cancer Institute*, *71*(4), 741–747.
9. TCC. (2020). SH-SY5Y (CRL-2266) [Cell line]. https://www.atcc.org/products/crl-2266
10. Kaya, Z.B., Santiago-Padilla, V., Lim, M. *et al.* Optimizing SH-SY5Y cell culture: exploring the beneficial effects of an alternative media supplement on cell proliferation and viability. *Sci Rep* **14**, 4775 (2024). https://doi.org/10.1038/s41598-024-55516-5
11. Kovalevich, J., & Langford, D. (2013). Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods in molecular biology (Clifton, N.J.)*, *1078*, 9–21. https://doi.org/10.1007/978-1-62703-640-5_2

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ABSTRACT

Neurotransmitters are essential chemical messengers within the body's nervous system, responsible for facilitating communication between neurons and their target cells. These biomolecules play a critical role in regulating a wide array of physiological processes, including the heartbeat, digestion, mood regulation, and muscle movement [1][2]. Given their importance in maintaining homeostasis and enabling brain function, neurotransmitters are a focal point in both neuroscience and pharmacological research. The increasing global emphasis on sustainability and environmental responsibility has prompted researchers to explore green chemistry approaches for synthesizing biologically significant molecules, including neurotransmitters [3][4]. This dissertation investigates the green synthesis of neurotransmitters and their functional analogs, emphasizing the use of environmentally benign reaction conditions to minimize ecological harm and the generation of toxic byproducts. By leveraging innovative techniques, natural reducing agents, and mild reaction parameters, the study aims to develop more sustainable pathways for neurotransmitter production [4]. Comprehensive characterization of the synthesized compounds was performed using Nuclear Magnetic Resonance (NMR) spectroscopy to confirm their structural integrity and ensure their biological relevance. Once characterized, these compounds are subjected to cell-based assays, such as the MTT assay, to evaluate their bioactivity and potential therapeutic effects. The research also explores the broader applications of green-synthesized neurotransmitters in neuropharmacology and biosensing [3][5], highlighting their enhanced compatibility with biological systems and improved therapeutic efficacy compared to traditionally synthesized counterparts. Ultimately, this work demonstrates that green synthesis not only offers a viable alternative to conventional production methods but also fosters more sustainable advancements in neurochemistry and biomedical research. By reducing environmental impact and improving the safety profile of neurotransmitter synthesis, green chemistry is paving the way for innovative solutions in neuroscience and drug development.

Keywords: Neurotransmitters; Serotonin; Green Synthesis; Blood Brain Barrier; Eco friendly; New Pharmacophore.

INTRODUCTION

Neurotransmitters are chemical substances that play an important role in transmitting signals across synapses, enabling communication between neurons and their target tissue sand that are synthesized in neurons as a result for some of its precursor substances to go through an enzymatic process [6]. Depending on the type of neurotransmitter, either the cell body or the presynaptic terminal may act as the production [1][7]. An example is the conversion of the amino acid tyrosine to dopamine, which involves enzymatic processes by tyrosine hydroxylase and aromatic L-amino acid decarboxylase, resulting in excitation, inhibition, or functional modification [7]. The Central Nervous System (CNS) utilize neurotransmitters to regulate various sensory, motor, and integrative neuronal messages, thereby affecting various bodily functions such as emotions, thoughts, memories, movements, and sleep patterns [6] Until now, scientists have identified over 100 neurotransmitters, with some of the most significant ones being acetylcholine, norepinephrine, dopamine, gamma-aminobutyric acid (GABA), glutamate, serotonin, and histamine [6][7]. Based on the functional effect, these chemical messengers can be broadly classified Some major types are: excitatory, inhibitory, and modulatory neurotransmitters. For example, excitatory neurotransmitters like glutamate stimulate the transmission of signals, while inhibitory neurotransmitters like GABA and glycine suppress signal transmission and lastly, Modulatory neurotransmitters, such as dopamine and serotonin, that influence the actions of other neurotransmitters and are essential for managing a number of physiological processes. Dysregulation of neurotransmitter activity has been implicated in various neurological and psychiatric disorders, like Alzheimer's disease, Parkinson's disease, depression, anxiety disorders, and schizophrenia. One such important neurotransmitters is Serotonin, which found in the brainstem and gastrointestinal tract [7]. Serotonin has various complicated role in mood regulation, sleep-wake cycles, appetite, memory, temperature regulation, pain perception, and emotional response. Serotonin is an inhibitory neurotransmitter and tends to cause calmness by the reduction of neuronal excitability. But there are some problems associated with serotonin, such as Serotonin which is large, polar molecule which have molecular weight approximately 176 Daltons, and contains multiple hydroxyl(-OH) groups, which contribute to its polarity, on the other side Blood Brain Barrier is designed to allow only small, non-polar or lipophilic molecules to pass through easily, hence serotonin cannot diffuse across the lipid bilayer of endothelial cells that form the Blood Brain Barrier [8][9]. Along with other limitation, including lack of selectivity, poor bioavailability, and various side effect. This research aimed to address these challenges by developing a Pharmacophore that could more effectively mimic serotonin's effects. The primary compounds utilized in this reaction was isatin and amines as starting materials.

AIMS AND OBJECTIVES

I. To develop Environmentally friendly, safe and effective therapeutic agents that can enhance neurotransmitter function or mimic their action in the treatment of neurodegenerative diseases,

- one aspect of my research is to synthesis by using green chemistry approach, emphasizing environmental sustainability
- This method helps in minimizing chemical waste and need for extensive purification steps.
- During the reaction copper is used as a catalyst, which was recovered and reused, promoting resource conservation and sustainable practice.

II. To Design and synthesize a Pharmacophore that can effectively cross blood brain barrier.

DESIGNING ASPECT

Figure 1: Structures of Serotonin, Tryptamine and D-Tryptophan

Serotonin's structure includes an indole ring system, to this indole core a hydroxyl group (-OH) is attached at carbon in the 5-position [10]. In contrast, tryptophan, an essential amino acid in the body, that serves as a precursor to serotonin, and Tryptamine, an aminoalkylindole consisting of indole having a 2-aminoethyl group at the 3-position, can easily cross the Blood Brain Barrier. Therefore, comparing the structure of serotonin with tryptophan and tryptamine compounds gives a hint that the presence of the hydroxyl group may hinder its ability to cross the Blood Brain Barrier [11].

For this thesis, we have chosen Two Bromo-Substituted Isatin Moiety (7-bromo Isatin and 5-Bromo Isatin) which have an indole ring (similar structure like serotonin) and two active centers for amine incorporation, one is Bromide center and another one is Ketone center which are shown in Figure 2. And amines that will react respectively are N, N-Dimethylaniline and 4-picolylamine. These selected amines are expected to form a stable product, that further modifies the original indole structure upon reaction with the Isatin Moieties thus enhancing the potential to cross the Blood Brain Barrier.

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MATERIALS AND METHOD

I. COMPOUND A:

- CHEMICALS AND REAGENTS: 7-Bromoisatin(7-bromo-1H-indole-2,3-dione); N, N-Dimethylaniline; Copper Nanoparticles; Distilled Water; Silica Gel; Hexane; Ethyl Acetate; Methanol; Chloroform.
- EQUIPMENT AND APPARATUS: Round Bottom Flask; Magnetic Beads; Spatula; Condenser; Magnetic Stirrer (or Magnetic Rotary Stirrer); Voltage Regulator; Tulu Pump; Silicone Oil Bath; Rotary Evaporator; Chromatography Columns; Funnels; Conical.

- REACTION:

Figure 3: Reaction of 7-Bromo Isatin and N, N-Dimethylaniline

- PROCEDURE:

Weighing Chemicals: Using weighing machine 1.5 mmol of 7-Bromoisatin which weigh about 339 mg is taken and using pipette we took 3 mmol of N, N-Dimethylaniline which measured about 375 µl.
Reaction Setup: Weighed chemicals which are carefully measured out are then transferred into a round-bottom flask, along with the catalyst, copper powder and Distilled water. Following the preparation of the mixture, the round-bottom flask containing the chemicals, copper nanoparticles, and distilled water is set up for heating under reflux conditions which involves connecting the round-bottom flask to a condenser. .

The round-bottom flask, which is connected to the condenser, is then placed in a silicon oil bath which is kept above Magnetic Stirrer Plate which provides continuous mixing and the oil bath is connected to a temperature regulator, which is used to maintain the temperature of the oil bath at a specific, constant value. In this procedure, the temperature is regulated around 120°C. The reaction is continued 48 hours.
Crude Preparation (Workup): After 48 hours when, the reaction is completed, the remaining water in the RBF is completely evaporated using rotary evaporator. After drying, we perform an extraction to separate the product from solid copper nanoparticles. For separation, we pour a polar solvent such as ethyl acetate, methanol, or chloroform. After adding the solvent, we keep the RBF still in the holder and then pour off the upper layer, separating it from the lower sediments of copper nanoparticles, into an Evaporating dish. We repeat this process 3 to 4 times and later add silica gel to create a slurry and leave it until it gets dry and form powdery.
Column Chromatography Setup and Purification: Once the column is cleaned, we secure it vertically using a ring stand. Next, to pack the stationary phase, we suspend silica gel in hexane and pour the slurry into the column until we reach desired hight. Once it reached, we load our sample onto the top of the packed column. Once the column is ready, open the pinch clamp (or stopcock) to allow the mobile phase to flow through it slowly, maintaining constant wetness in the column. The eluates are collected in conical flasks, which are then poured into a round-bottom flask. The collected eluate is then evaporated using a rotary evaporator, leaving the solid or liquid product behind. After repeating the elute collection process at a particular solvent percentage for some time, the solvent percentage is gradually increased and it's continued until we start getting solid or liquid product.
Monitoring Progress of the Reaction: To monitor the progress of the reaction, the product is mixed with a solvent such as ethyl acetate or methanol. A small capillary is then used to place a small dot on a TLC plate, which is subsequently kept in a mobile phase jar. Finally, the plate is examined under UV light to determine whether the desired compound has been separated.
Sample collection: The solid or liquid product that we obtained is then collected in culture tubes and kept for several days in a dark place to allow for crystal formation. A few crystals are then collected in a tube for further study of the product's structure. The remaining crystals are weighed and dissolved in Dimethyl Sulfoxide (DMSO) for cell testing.

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CELL TESTING OF COMPOUND A

o CELL LINE DESCRIPTION: SH-SY5Y cells, derived from neuroblastoma cell line which were subcloned from the parental SK-N-SH cell line, which originated from a bone marrow biopsy of a four-year-old female [12]. These cells display a neuroblast-like morphology, growing as both adherent and floating clusters, and can be differentiated into neuron-like cells using compounds such as retinoic acid and neurotrophins [13],[14]). SH-SY5Y cells are used worldwide as an in-vitro model for neuronal function and differentiation, they can be characterized by their ability to proliferate in culture, express immature neuronal markers in the undifferentiated state, and upon differentiation, exhibit mature neuronal markers and cease proliferation [14][15]. Their sensitivity to culture conditions and slow proliferation rate requires careful handling, but their biochemical similarity to human dopaminergic neurons and versatility in experimental neuroscience make them a key tool for research [14].

o METHODOLOGY
Thawing: SH-SY5Y cells were retrieved from cryopreservation, for which the frozen vial was quickly removed from liquid nitrogen and placed immediately in a 37°C water bath. Gentle agitation was applied to ensure rapid and even thawing, taking care to remove the vial as soon as the contents were completely liquid to minimize exposure to potentially cytotoxic DMSO. Then the thawed cells were transferred into a sterile conical tube containing growth medium. After 24 hours, the medium was replaced to remove non-viable cells and any remaining DMSO, ensuring optimal cell health and attachment.
Splitting of SH-SY5Y Cells: After 2 to 3 days the old growth medium was aspirated, and the cell monolayer was gently rinsed with 1X PBS to remove residual serum and cell debris.
After aspirating the PBS, 500ul-1ml 0.25% trypsin-EDTA solution was added to the flask. The flask was incubated at 37°C for 2–3 minutes, with periodic gentle tilting to aid cell detachment. The cell suspension was triturated gently with a pipette to ensure a single-cell suspension and then centrifuged it at 1000g for 5 minutes. The supernatant was removed, and the cell pellet was resuspended in 1ml DMEM with 10%FBS supplement. 10ul of the solution and 50ul of trypan blue was added to make 6X dilution. 10ul of the solution was taken Then the cells counted using a haemocytometer.
Freezing of SH-SY5Y Cells: Cells when reached to 85-95% confluency, were aliquoted into cryovials at a density of 2-6 million cells per vial. The cryovials were pre-cooled at 4°C for 10 minutes, then placed at –20°C for an additional 10 minutes to allow gradual temperature reduction. The vials were then transferred to a –80°C freezer for 24 hours before being moved to liquid nitrogen for long-term storage.
Cell Seeding and Treatment: In 96-well plate, they were seeded around 10,000 to 20,000 cells per

well. Once the cells reached a desired confluency of around 50-60%, Cells in 6-well plates were divided into 4 groups:
- Naive control [only media change without FBS],
- Vehicle Control [0.1% DMSO in the DMEM without FBS],
- 1 µM of Compound A [DMEM without FBS], and
- 10 µM of Compound A [DMEM without FBS]. (The use of serum-free media (DMEM without FBS)

After 48 hours of the treatment, 10 µl of MTT solution (5 mg/ml) was added to each well (final conc. 0.45 mg/ml). The solution was then incubated for 1-4 hrs at 37° C. 100 µl DMSO was added to each well for dissolving the formazan crystals. The solution was mixed properly to ensure the complete solubilization. Absorbance was measured at 570 nm.

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II. COMPOUND B:

- CHEMICALS AND REAGENTS: 5-Bromoisatin; 4-Picolylamine; Copper Nanoparticles; Distilled Water; Silica Gel; Hexane; Ethyl Acetate; Methanol; Chloroform.
- EQUIPMENT AND APPARATUS: Round Bottom Flask; Magnetic Beads; Spatula; Condenser; Magnetic Stirrer (or Magnetic Rotary Stirrer); Voltage Regulator; Tullu Pump; Silicone Oil Bath; Rotary Evaporator; Chromatography Columns; Funnels; Conical.

- REACTION:

- PROCEDURE:
Weighing Chemicals: 1.5 mmol of 5-Bromoisatin is weighed about 329 mg is taken and using pipette we took 3 mmol of 4-Picolylamine which measured about 375 µl.
Reaction Setup: Weighed chemicals which are carefully measured out are then transferred into a round-bottom flask, along with the catalyst, copper powder and Distilled water. Following the preparation of the mixture, the round-bottom flask containing the chemicals, copper nanoparticles, and distilled water is set up for heating under reflux conditions which involves connecting the round-bottom flask to a condenser. .

The round-bottom flask, which is connected to the condenser, is then placed in a silicon oil bath which is kept above Magnetic Stirrer Plate which provides continuous mixing and the oil bath is connected to a temperature regulator, which is used to maintain the temperature of the oil bath at a specific, constant value. In this procedure, the temperature is regulated around 120°C. The reaction is continued 12 hours.
Crude Preparation (Workup): 12 hours, After the reaction is over the remaining water in the RBF is completely evaporated using rotary evaporator. After drying, we perform an extraction to separate the product from solid copper nanoparticles. For separation, we pour a polar solvent such as ethyl acetate, methanol, or chloroform. After adding the solvent, we keep the RBF still in the holder and then pour off the upper layer, separating it from the lower sediments of copper nanoparticles, into an Evaporating dish. We repeat this process 3 to 4 times and later add silica gel to create a slurry and leave it until it gets dry and form powdery.
Column Chromatography Setup and Purification: Once the column is cleaned, we secure it vertically using a ring stand. Next, to pack the stationary phase, we suspend silica gel in hexane and pour the slurry into the column until we reach desired hight. Once it reached, we load our sample onto the top of the packed column. Once the column is ready, open the pinch clamp (or stopcock) to allow the

mobile phase to flow through it slowly, maintaining constant wetness in the column. The eluates are collected in conical flasks, which are then poured into a round-bottom flask. The collected eluate is then evaporated using a rotary evaporator, leaving the solid or liquid product behind. After repeating the elute collection process at a particular solvent percentage for some time, the solvent percentage is gradually increased and it's continued until we start getting solid or liquid product.
Monitoring Progress of the Reaction: To monitor the progress of the reaction, the product is mixed with a solvent such as ethyl acetate or methanol. A small capillary is then used to place a small dot on a TLC plate, which is subsequently kept in a mobile phase jar. Finally, the plate is examined under UV light to determine whether the desired compound has been separated.
Sample collection: The solid or liquid product that we obtained collected for a Nuclear Magnetic Resonance or NMR tube for further study of the product's structure.

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RESULTS AND DISCUSSIONS

I. COMPOUND A:

• Characterization of compound [A] using 1H-NMR (600MHz):

The ^{1}H NMR spectrum provides information:
o Aromatic Region: A complex series of multiplets is observed between approximately δ 6.6 ppm and 7.5 ppm. his region is characteristic of aromatic protons. The molecule has an indole ring (containing 4 aromatic protons), a brominated benzene ring (containing 3 aromatic protons), and two N-methylated benzene rings (each containing 2 aromatic protons due to symmetry).
o Peak "a": A signal is observed around δ 10.80 ppm, integrating approximately 1H. This chemical shift is characteristic of an amide proton (N-H).
o Peak "b": A sharp singlet is observed at approximately δ 3.49 ppm, with a large integration of 12.00 H. This chemical shift represents the presence of aliphatic protons.

• Characterization of compound [A] using 13C-NMR (150MHz):

Figure 6: ^{13}C NMR spectrum of Compound A

The ^{13}C NMR spectrum provides information:
o Carbonyl Carbon (Peak "a"): A singlet at δ 179.58 ppm: This signal is significantly downfield and is characteristic of a carbonyl carbon (C=O).
o Aromatic Carbons: A cluster of signals appears in the region between approximately δ112.60 ppm and 149 ppm. This region is typical shows presence for aromatic carbons.
o Peak "c": It represent a quaternary carbon.
o N-CH3 Carbons (Peak "b"): A peak at 62.10 ppm shows a chemical shift suggests a carbon attached to a nitrogen atom in one of the N-methylated benzene rings.

• RESULT OF CELL TESTING:
In the Figure 7 bellow, marked (i) is the Control; (ii) is the vehicle control; and in the Figure 8 bellow, marked (iii) is with 100nM concentration of compound A; (iv) is with1μM concentration of compound A.

The results from the cell testing showed a strong formation of neuronal networks and an increase in synaptic neurons across four different setups: Control, Vehicle Control, one with a 100nM concentration, and another with a 1μM concentration of compound A after 48 hours. Thus, it shows a positive effect on neural connectivity and function. Observing well-formed neuronal networks is essential for evaluating the neurodevelopmental effects of compounds in vitro. At the same time, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a well-known method for measuring cell metabolic activity and viability, confirmed that Compound A is not cytotoxic. With these promising in vitro results, Compound A is considered ready for further in vivo studies. These studies will shed light on its pharmacokinetics, pharmacodynamics, and overall therapeutic potential in a living organism

(i) Control (ii) Vehicle Control
Figure 7: Microscopic Images of SH-SY5Y neuroblastoma cells after 48 hours
(i) In Control setup: Cells were subjected to media change only. After 48 hours they are still exhibiting Normal Morphology. (ii) Vehicle Control: After 48 hours the appearance of these cells seems similar to control, indicating the presence of DMSO does not have a significant effect on cell morphology

(iii) 100nM concentration (iv) 1μM Concentration

Figure 8: Microscopic Images of SH-SY5Y cells 48 hours after treatment with Compound A. Synthesized Compound A was tested on SH-SY5Y Cells at two different concentrations of (iii) 100nM and (iv) 1μM Concentration. At both Concentrations, the cell, induced significant neuronal network formation and extensive neurite outgrowth, similar to the effects typically observed with serotonin.

II. COMPOUND B:

• Characterization of compound [B] using 1H-NMR (600MHz):

The ^{1}H NMR spectrum provides information:
o Aromatic Region: A cluster of signals between δ 6.4 and 8.3 ppm. The integrated region shows around 7 aromatic protons.
[] The signals at δ 8.290, 7.744, 7.737, 7.344, 7.323, 7.316 ppm likely correspond to the protons on the pyridyl ring (the six-membered ring containing nitrogen).
[] The signals at δ 7.029, 7.025, 6.467 ppm likely correspond to the protons on the bromo-substituted indole ring system.
o Peak "a": A broad signal at δ 10.03 ppm. This chemical shift is characteristic of an indole N-H proton, integrating one proton, consistent with the single N-H in the indole ring
o Peak "b": A signal is observed at approximately δ 5.52 ppm, with an integration of 1H. This chemical shift suggests a proton attached to a carbon that is bonded to an electronegative atom, like nitrogen. This is a methine proton (C-H) on the indole ring adjacent to the nitrogen and the bromine-substituted benzene part.
o Peak "c": A signal is observed at approximately δ 3.90 ppm, with an integration approximately 1H. This chemical shift also suggests the presence of Ethylenic protons.

• Characterization of compound [B] using 13C-NMR (150MHz)

Figure 10: ^{13}C NMR spectrum of Compound B

The ^{13}C NMR spectrum provides information:

o Carbonyl Carbon (peak a): A prominent peak is visible at δ 177.630 ppm: This signal is significantly downfield and is characteristic of a carbonyl carbon (C=O).
o Aromatic Carbons: The cluster of signals between 111 ppm and 150 ppm corresponds to the carbon atoms in the two aromatic rings (the bromo-substituted indole and the pyridyl ring). Each unique carbon atom in these rings will give rise to a separate signal. The number of signals in this region (11 distinct peaks are clearly visible) is consistent with the 11 non-equivalent carbon atoms in the aromatic system.
o Aliphatic Carbons (peak b and peak c): Two Peaks around δ 63.872 ppm and δ 58.731 ppm indicates the presence of Aliphatic carbon.

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6. CONCLUSION:

For Compound A, the combined 1H and 13C NMR data confirm the compound a is 7-bromo-3,3-bis[4-(dimethylamino)phenyl]-indolin-2-one. Further cell testing demonstrate that Compound A promotes the formation of neuronal networks and increases synaptic neuron counts, indicating a positive effect on neural connectivity and function. And the MTT assay further confirms that Compound A is not cytotoxic.
For Compound B, the combined 1H and ^{13}C NMR data confirm the compound B is (E)-5-bromo-3-(piperidin-4-ylmethylene)aminoindolin-2-one.
With this I would like to conclude that the both compounds have been thoroughly characterized at the molecular level, and Compound A has shown promising primary biological activity enhancing neuronal network formation without cytotoxicity. Therefore, these findings provide a strong basis for advancing both compounds to further studies, including in vivo evaluation of their pharmacokinetics, pharmacodynamics, and therapeutic potential in neurodevelopmental and neuroprotective contexts.

No Plagiarism Found